Published in: Clinical & Experimental Allergy (2006), vol. 36, iss. 5, pp. 563-567
Status: Postprint (Author's version)

Is the neutrophil a worthy target in severe asthma and chronic obstructive pulmonary disease?

R. Louis* and R. Djukanovic†
** Department of Pneumology and Center for Biointegrative Genoproteomic (CBIG), CHU Sart-Tilman, University of Liege, Liege, Belgium and †Allergy and Inflammation Research, Division of Infection, Inflammation and Repair, General Hospital University of Southampton, UK*

Both asthma and chronic obstructive pulmonary disease (COPD) are recognized as chronic inflammatory airway diseases. Over the past 20 years, considerable effort has gone into elucidating the complex cell and mediator interactions taking place in airways of patients with these diseases. Traditionally, asthma has been viewed as an eosinophilic disease, while neutrophils, together with macrophages, have been seen as being key inflammatory cells in COPD [1]. However, recent studies have blurred this rather simple distinction, with studies showing that a proportion of asthmatics, predominantly with severe asthma, have raised neutrophils in their airway lumen [2] and, interestingly, in their bronchial wall [3, 4]. Similarly, studies in COPD show that the numbers of airway eosinophil may increase in stable patients with sign of mast cell activation [5] and during exacerbations [6].

In a recent issue of *Clinical and Experimental Allergy,* Beeh and Beier provide a comprehensive review of the role of neutrophils in severe asthma and COPD and discuss potential targets of anti-neutrophil treatment [7]. This editorial discusses further the role of neutrophils in severe asthma and COPD and the critical importance of choosing the right outcomes when assessing the efficacy of new anti-inflammatory drugs in severe asthma and COPD.

Neutrophils in severe asthma: key players or mere bystanders?

Asthma has long been considered to be an eosinophilic bronchitis, which is associated with bronchial hyper-responsiveness. The beneficial effects of inhaled corticosteroids in asthma have commonly been thought to be related to the ability of these drugs to dramatically reduce the airway eosinophilia [8]. However, recent studies have shown that despite a dramatic reduction in blood and airways eosinophils by anti-IL-5, the anticipated protection against allergen challenge and the associated increase in bronchial hyper-responsiveness is not seen in patients with mild asthma [9]. Furthermore, in a limited study, this type of treatment has not been shown to improve forced expiratory volume in 1 s (FEV_1) and symptoms in severe asthma [10]. With the finding that anti-IL-5 treatment fails to completely deplete eosinophils from the bronchial mucosa [11], the debate about the role of eosinophils in asthma has remained open but it has also become clear that more work has to be directed at other effector cells. At the same time, the recognition that some asthmatics, particularly those who have severe disease [2, 3, 12] and are resistant to corticosteroids [13], have raised neutrophil counts in their airways has led to suggestions that neutrophils might be a more valid target than eosinophils when attempting to bring asthma under control. In addition, the fact that activated neutrophils may release a large array of inflammatory mediators, oxygen radicals and proteases has lent support to their involvement in the intense airways inflammation and remodelling found in severe asthma [14] (Fig. 1). Thus, elegant studies have shown that raised sputum neutrophil counts are associated with irreversible loss in lung function in asthmatics, as reflected by FEV_1 values measured after completion of a 14-day course of oral corticoids and high-dose nebulized salbutamol [15].

Despite the increasing evidence to support a role for neutrophils in asthma, the mere increase in their numbers does not imply that they are pivotal in severe asthma. The fact that severe asthma with irreversible lung function impairment is associated with high airway neutrophilia also does not imply that neutrophils are involved in airway remodelling. Rather, this association might indicate that remodelled airways, whatever the mechanisms leading to remodelling may be, provide a suitable environment that favours neutrophil recruitment or survival [16]. Furthermore, patients in this category are usually on inhaled corticosteroids, which could prolong neutrophil survival [17], making the association circumstantial rather than one of cause and effect.

An important question when seeking to identify a role for a cell in disease is whether the presence of increased numbers necessarily implies increased activation and if so does this have deleterious effects or is it a beneficial, protective reaction. Of relevance to the question of the pathogenetic role of neutrophils is the observation that reducing airway lumen neutrophil numbers by leuko-triene B_4 (LTB_4) antagonists [18] does not reduce the fall in lung function following allergenic exposure. In that respect, the story is reminiscent of the effects of depleting

blood and airway eosinophils with anti-IL-5. When looking at the available evidence, it is also important to consider the compartment where cells are sampled. The majority of studies, which have highlighted the role of neutrophils in severe asthma have been based on the analysis of sputum or bronchoalveolar lavage, both of which sample the lung air spaces. In contrast to eosinophils, where a good correlation exists between sputum and bronchial eosinophil counts, a similar association has not been seen for neutrophils [19]. Therefore, a high neutrophilia in the airway lumen, as usually shown in chronic severe asthma [20] and in some asthma exacerbations [21], does not necessarily imply that the bronchial wall is equally infiltrated with neutrophils. The potential remodelling effect of an inflammatory cell within the airway lumen, however, is likely to be less than that of the same cell located within the bronchial wall. Fewer biopsy studies have been performed in severe asthma. Intense bronchial mucosal neutrophilic inflammation is not a characteristic finding in all chronic severe asthmatics who fail to be adequately controlled by inhaled or oral corticosteroids [22]. When significant neutrophilic infiltration was found [4], it was associated with prominent infiltration with eosinophils, mast cells and CD3 T cells, making it difficult to conclude definitively whether neutrophils were the determinants of asthma severity.

Other potential cell targets in severe asthma

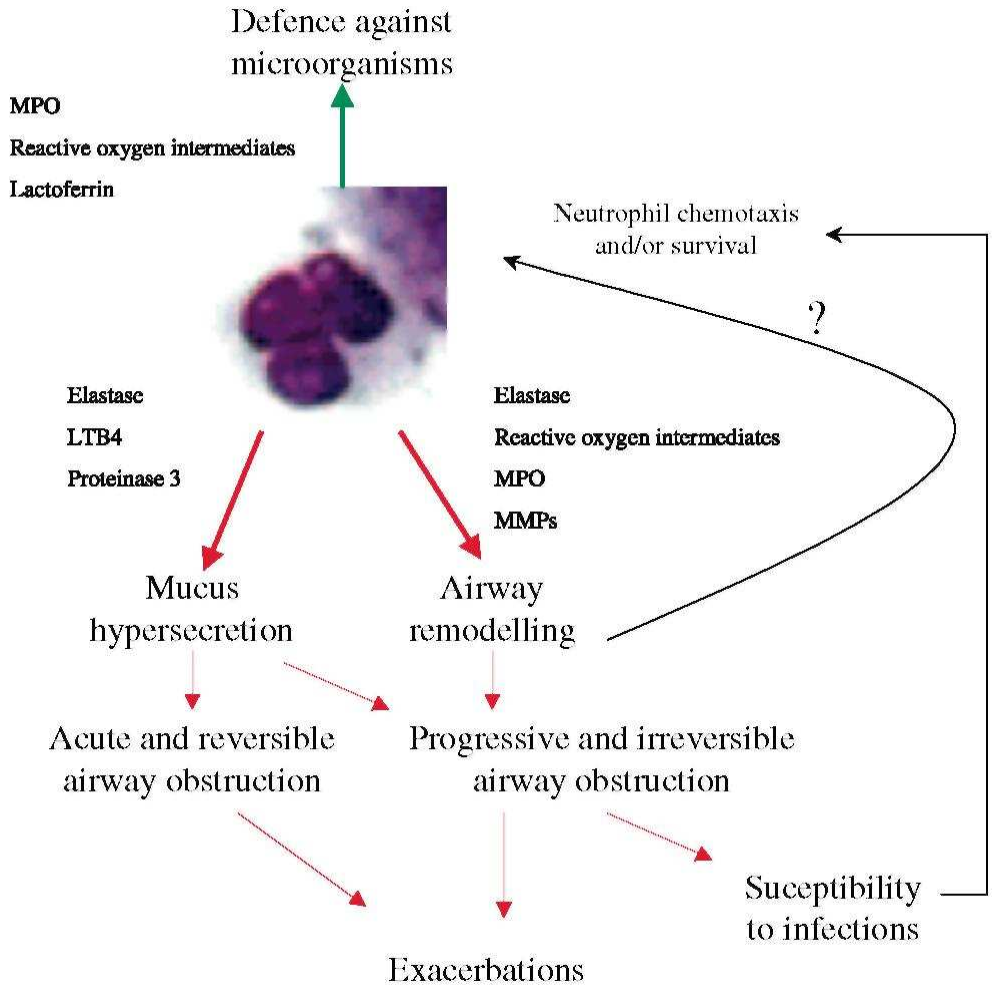
There are several other cell types that deserve further attention as potential targets in the clinical setting of severe asthma. Recent intriguing data have indicated that bronchial mucosal $CD8^+$, rather than $CD4^+$, T cells, are associated with the lung function decline in asthma [23], suggesting a role for these cells in airways remodelling. A potential role for $CD8^+$ T cells has also been recently highlighted as possibly precipitating asthma death during viral infection [24]. Looking beyond inflammation, there is now little doubt that the structural cells that constitute the airways are important in asthma [25]. Among these, airway smooth muscle cells, have long been known to have an altered phenotype. Because asthma is a disease where lung function can change dramatically and respond very promptly to β_2-agonists, the evidence for the role of smooth in asthma is conclusive. The smooth muscle layer is considerably thickened in severe asthma [22] prompting a lot of research into the mechanisms leading to smooth muscle proliferation [26]. Of particular interest is the recent demonstration that the airway smooth muscle layer in asthma is infiltrated by mast cells [27] and that this is a major feature that distinguishes asthma and eosinophilic bronchitis, which is not associated with bronchial hyper-responsiveness. This tight spatial relationship between mast cells and smooth muscle may be of critical importance not only in the events triggering acute bronchos-pasm following allergen exposure, but may also be important in the genesis of smooth muscle hypertrophy/ hyperplasia.

Targeting tumour necrosis factor-α rather than neutrophils in severe asthma

Rather than trying to deplete the airways of their inflammatory cells, some therapeutic strategies aim to counteract cytokines released by these cells on the assumption that these might be pivotal to asthma pathogenesis. After a series of disappointing results obtained with anti-T-helper cells type 2 (Th2) cytokines (anti-IL-5 or anti-IL-4), current research has turned towards blocking non-specific pro-inflammatory cytokines such as TNF-α which are classically associated with neutrophilic inflammation. This strategy has proved to be efficient in other chronic inflammatory diseases such as rheumatoid arthritis [28] and Crohn disease [29]. Anti-TNF-α is presented by Beeh and Beier[7] as an anti-neutrophilic strategy. Two very recent studies have shown that targeting TNF-α in severe corticosteroid dependent asthma with etanercept, a p75 TNF receptor-IgG fusion protein, that binds to TNF-α and TNF-β, could benefit patients in terms of symptom control and quality of life [30, 31]. Interestingly, this improvement was found to be independent of any reduction in airways neutrophil counts but was linked to the baseline expression of membrane-bound TNF-α by peripheral-blood monocytes [31]. This suggests that the detrimental up-regulation of the TNF-α axis may be independent of airways neutrophils in severe asthma. Even more surprising and interesting, is the fact that etanercept rapidly and convincingly reduced the extent of methacholine bronchial hyper-responsiveness [30, 31], lending support to a possible direct action of TNF-α on smooth muscle. Together with the ability of inhaled TNF-α to induce airway hyper-responsiveness [32] and the recent demonstration that airway smooth muscle is typically infiltrated with mast cells in asthma, these observations emphasize the importance of the localization of inflammation within the wall [33]. Based on these observations, it is also tempting to speculate that TNF-α released by mast cells in the vicinity of airway smooth muscle profoundly affects the behaviour of the latter. This would fit the hypothesis according to which asthma is a smooth muscle inflammation in which mast cell-derived mediators/cytokines play a prominent role.

Published in: Clinical & Experimental Allergy (2006), vol. 36, iss. 5, pp. 563-567
Status: Postprint (Author's version)

Fig. 1. *Role of neutrophil in chronic obstructive airway diseases. Whereas the green arrow represents a protective mechanism, events indicated by the red arrows are detrimental to the patients.*



Are neutrophils key cells leading to lung function decline in chronic obstructive pulmonary disease?

As far as COPD is concerned, emphasis has traditionally been placed on macrophages and neutrophils, and more recently $CD8^+$ T cells, as key cells orchestrating inflammation and mediating the tissue damage that characterizes the disease [34]. Of particular importance is the recognized ability of neutrophils to release oxygen radicals and elastase, which may be essential for the pathogenesis of bronchiolitis and emphysema, respectively. Furthermore, neutrophils appear to be a potent source of matrix metalloproteinases, the role of which has recently been highlighted in airway remodelling [35]. Therefore, neutrophil activation has the biochemical potential to initiate remodelling and speed up lung function decline in COPD. There is compelling evidence in the literature to support that view. In advanced COPD, sputum neutrophilia may account for up to 70-90% of the cells found in the airway lumen [36] and sputum neutrophilia is associated with greater airflow limitation as measured by both FEV_1 and the FEV_1/forced vital capacity ratio, together with an accelerated decrease in FEV_1 over a 15 year follow-up period [37]. In addition, a recent study of surgically resected lung tissue in a large number of COPD patients showed that the number of neutrophils in the small airways clearly increases in association with the reduction in FEV_1 [38] (Fig. 2). However, arguments have been put forward that neutrophils are more related to smoking itself rather than to airway obstruction and emphysema [39]. Additionally, there are examples in clinical medicine in which extensive and selective lung infiltration by neutrophils does not result in severe remodelling. One of those is pneumococcal pneumonia, which usually resolves completely after a short period of inflammation when the patient is appropriately treated with antibiotics. It is, therefore, reasonable to postulate that remodelling occurs after prolonged neutrophilic infiltration, and/or when neutrophils interact with other inflammatory cells. It may also be that the phenotype of the neutrophil is markedly different in chronic disease. In this respect, it is also important to emphasize that both in severe asthma [4] and COPD [38] neutrophilic infiltration coexists with infilitration with other inflammatory cells such as T and B cells,

eosinophils and macrophages.

Neutrophils and mucus production

Neutrophils rapidly traffic from blood (through airway wall) into the airway lumen where they contribute to defences against microorganisms [40]. In their review Beeh and Beier [7] briefly state that neutrophils may release products that stimulate mucus secretion. This interaction between neutrophils and mucous glands certainly deserves further attention. Indeed, diseases where sputum is produced in abundance, such as cystic fibrosis and bronchiectasis, are often associated with intense sputum neutrophilia [41, 42]. Furthermore, smokers with chronic bronchitis [43] and asthmatics [44] have neutrophil infiltration of their bronchial glands. These observations suggest that products secreted by neutrophils might play a critical role in mucus secretion. This is further supported by the fact that mediators released by neutrophils, such as LTB_4 [45], elastase and proteinase 3 [46], are potent stimuli for glandular secretion *in vitro* (Fig. 1). Both in severe COPD [38] and fatal asthma [47], there is convincing evidence of mucus retention in the airways which contributes to airway lumen obliteration and, thereby, to airflow impairment. In addition chronic mucus hyper-secretion is associated with accelerated lung decline and risk of hospitalization [48]. Therefore, therapeutic strategies that would aim to regulate the amount and viscosity of mucus secreted in the airways would be of great value in the management of both severe asthma and COPD. Careful analysis of interactions between neutrophils and airway glands might lead to new pharmacological tools to control airway secretion in the future.

The importance of choosing the right outcome in severe asthma and COPD

Strategies that dramatically reduce eosinophils in asthma, such as the use of monoclonal antibody directed against IL-5, have probably been dismissed too early. Better selection of patients - primarily those with persistent eosinophilia - and a better choice of outcome in the study design are needed. Given that eosinophils might have a particularly important role in asthma exacerbations, either directly or as markers of a risk of exacerbation [49], and in view of their possible role in COPD exacerbations [6], studies aimed at elucidating whether there is any benefit of reducing exacerbations by controlling eosinophil numbers by way of anti-IL-5 antibody treatment would be welcome. In the design of such studies care should be taken to select those asthmatics in whom high sputum (and perhaps blood) eosinophil counts persist despite treatment with high doses of inhaled corticosteroids. As pointed out by Beeh and Beier [7] there is not yet convincing evidence for a drug that selectively and consistently reduce neutrophilic inflammation. If such a drug endowed with selective anti-neutrophil activity, validated in a proof of concept study, were to be tested in severe asthma and COPD, exacerbation frequencies, the rate of lung function decline and possibly mortality should be chosen as primary outcomes. This would, of course, require long-term studies and large cohorts of patients. However, it should be borne in mind that clinical situations in which neutrophil numbers are markedly reduced put patients at risk of developing severe and potentially lethal infections.

References

1 Jeffery PK. Structural and inflammatory changes in COPD: a comparison with asthma. *Thorax* 1998; **53**:129-36.

2 Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 2000; **161**:9-16.

3 Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* 1997; **156**:737-43.

4 Wenzel SE, Schwartz LB, Langmack EL *et al.* Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; **160**:1001-8.

5 Louis RE, Cataldo D, Buckley MG *et al.* Evidence of mast-cell activation in a subset of patients with eosinophilic chronic obstructive pulmonary disease. *Eur Respir* J 2002; **20**:325-31.

6 Saetta M, Di Stefano A, Maestrelli P *et al.* Airway eosinophilia in chronic bronchitis during exacerbations. *Am J Respir Crit Care Med* 1994; **150**:1646-52.

7 Beeh KM, Beier J. Handle with care: targeting neutrophils in chronic obstructive pulmonary disease and severe asthma? *Clin Exp Allergy* 2006; **36**:142-57.

8 Djukanovic R, Wilson JW, Britten KM *et al.* Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992; **145**:669-74.

9 Leckie MJ, ten Brinke A, Khan J *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; **356**:2144-8.

10 Kips JC, O'Connor BJ, Langley SJ *et al.* Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003; **167**:1655-9.

11 Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003; **167**:199-204.

12 Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999; **160**:1532-9.

13 Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; **57**:875-9.

14 Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000; **161**: 1720-45.

15 Little SA, MacLeod KJ, Chalmers GW, Love JG, McSharry C, Thomson NC. Association of forced expiratory volume with disease duration and sputum neutrophils in chronic asthma. *Am J Med* 2002; **112**:446-52.

16 Sato E, Koyama S, Takamizawa A *et al.* Smoke extract stimulates lung fibroblasts to release neutrophil and monocyte chemotactic activities. *Am J Physiol* 1999; **277**:L1149-57.

17 Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J Immunol* 1995; **154**:4719-25.

18 Evans DJ, Barnes PJ, Spaethe SM, van Alstyne EL, Mitchell MI, O'Connor BJ. Effect of a leukotriene B4 receptor antagonist, LY293111, on allergen induced responses in asthma. *Thorax* 1996; **51**:1178-84.

19 Maestrelli P, Saetta M, Di Stefano A *et al.* Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoal-veolar lavage. *Am J Respir Crit Care Med* 1995; **152**:1926-31.

20 The ENFUMOSA Cross-Sectional European Multicentre Study of the Clinical Phenotype of Chronic Severe Asthma. European network for understanding mechanisms of severe asthma. *Eur Respir J* 2003; **22**:470-7.

21 Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 1995; **95**:843-52.

22 Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003; **167**:1360-8.

23 van Rensen EL, Sont JK, Evertse CE *et al.* Bronchial CD8 cell infiltrate and lung function decline in asthma. *Am* J *Respir Crit Care Med* 2005; **172**:837-41.

24 O'Sullivan SM. Asthma death, CD8+ T cells, and viruses. *Proc Am Thorac Soc* 2005; **2**:162-5.

25 Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003; **111**:215-25.

26 Hirst SJ, Martin JG, Bonacci JV *et al.* Proliferative aspects of airway smooth muscle. *J Allergy Clin Immunol* 2004; **114**:S2-S17.

27 Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; **346**:1699-705.

28 Maini RN, Elliott MJ, Brennan FM *et al.* Monoclonal anti-TNF alpha antibody as a probe of pathogenesis and therapy of rheumatoid disease. *Immunol Rev* 1995; **144**:195-223.

29 Targan SR, Hanauer SB, van Deventer SJ *et al.* A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's disease cA2 study group. *N Engl J Med* 1997; **337**:1029-35.

30 Howarth PH, Babu KS, Arshad HS *et al.* Tumour necrosis factor (TNF{alpha}) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. *Thorax* 2005; **60**:1012-8.

31 Berry MA, Hargadon B, Shelley M *et al.* Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl J Med* 2006; **354**:697-708.

32 Thomas PS, Heywood G. Effects of inhaled tumour necrosis factor alpha in subjects with mild asthma. *Thorax* 2002; **57**:774-8.

33 Brightling CE, Pavord ID. Location, location, location: micro-localisation of inflammatory cells and airway dysfunction. *Thorax* 2004; **59**:734-5.

34 Sutherland ER, Martin RJ. Airway inflammation in chronic obstructive pulmonary disease: comparisons with asthma. *J Allergy Clin lmmunol* 2003; **112**:819-27.

35 Cataldo DD, Gueders MM, Rocks N *et al.* Pathogenic role of matrix metalloproteases and their inhibitors in asthma and chronic obstructive pulmonary disease and therapeutic relevance of matrix metalloproteases inhibitors. *Cell Mol Biol (Noisy-le-grand)* 2003; **49**:875-84.

36 Culpitt SV, Maziak W, Loukidis S, Nightingale JA, Matthews JL, Barnes PJ. Effect of high dose inhaled steroid on cells, cytokines, and proteases in induced sputum in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; **160**:1635-9.

37 Stanescu D, Sanna A, Veriter C *et al.* Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996; **51**:267-71.

38 Hogg JC, Chu F, Utokaparch S *et al*. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004; **350**:2645-53.

39 Saetta M, Di Stefano A, Maestrelli P, Mapp CE, Ciaccia A, Fabbri LM. Structural aspects of airway inflammation in COPD. *Monaldi Arch Chest Dis* 1994; **49**:43-5.

40 Hiemstra PS, van Wetering S, Stolk J. Neutrophil serine proteinases and defensins in chronic obstructive pulmonary disease: effects on pulmonary epithelium. *Eur Respir J* 1998; **12**: 1200-8.

41 Loukides S, Bouros D, Papatheodorou G, Lachanis S, Panagou P, Siafakas NM. Exhaled H(2)0(2) in steady-state bronchiectasis: relationship with cellular composition in induced sputum, spirometry, and extent and severity of disease. *Chest* 2002; **121**:81-7.

42 Sagel SD, Kapsner R, Osberg I, Sontag MK, Accurso FJ. Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. *Am J Respir Crit Care Med* 2001; **164**:1425-31.

43 Saetta M, Turato G, Facchini FM *et al.* Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. *Am J Respir Crit Care Med* 1997; **156**:1633-9.

44 Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; **57**:677-82.

45 Cardell LO, Agusti C, Takeyama K, Stjarne P, Nadel JA. LTB(4)-induced nasal gland serous cell secretion mediated by neutrophil elastase. *Am J Respir Crit Care Med* 1999; **160**:411-4.

46 Witko-Sarsat V, Halbwachs-Mecarelli L, Schuster A *et al.* Proteinase 3, a potent secretagogue in airways, is present in cystic fibrosis sputum. *Am J Respir Cell Mol Biol* 1999; **20**:729-36.

47 Carroll N, Carello S, Cooke C, James A. Airway structure and inflammatory cells in fatal attacks of asthma. *Eur Respir J* 1996; **9**:709-15.

48 Vestbo J, Prescott E, Lange P. Association of chronic mucus hypersecretion with FEV1 decline and chronic obstructive pulmonary disease morbidity. Copenhagen City Heart Study Group. *Am J Respir Crit Care Med* 1996; 153:1530-5.

49 Green RH, Brightling CE, McKenna S *et al.* Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *lancet* 2002; **360**:1715-21.